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Zebrafish bioassay-guided microfractionation combined with CapNMR a comprehensive approach for the identification of anti-inflammatory and anti-angiogenic constituents of Rhynchosia viscosa

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Abstact

Zebrafish have recently emerged as an attractive *i*n vivo system for functional genomics and drug discovery [1]. Because of their small size, rapid development, optical transparency, and high genetic, physiologic, and pharmacologic similarity with humans, zebrafish embryos and larvae are also an ideal model for natural product discovery. As zebrafish bioassays require only microgram amounts of crude extracts, chromatographic fractions, and pure compounds, we are developing a zebrafish-based natural product discovery platform that takes advantage of modern techniques for the isolation and structural elucidation of natural products, such as UHPLC-TOF-MS profiling, LC-MS microfractionation and subsequent capillary NMR characterization [2]. An in vivo, zebrafish-based anti-inflammatory screen of methanolic extracts of East African medicinal plants resulted in the identification of *R. viscosa*, which revealed potent inhibition of leukocyte migration after larval tail transection in the presence of bacterial lipopolysaccharides. This extract also displayed anti-angiogenic activity in transgenic zebrafish with vasculature-specific expression of GFP. Intriguingly, *R. viscosa* is used by traditional healers in Tanzania for the treatment of inflammatory skin disorders and insect bites, corroborating our findings in zebrafish. Microfractionation of the crude methanolic extract was performed, and individual fractions tested for bioactivity in zebrafish. One highly active fraction was subjected to HR-ESI-MS and CapNMR analysis, resulting in the identification of genistein – a known inhibitor of inflammation and angiogenesis. These results indicate the potential of zebrafish bioassay-guided microfractionation, in combination with sub-milligram NMR techniques, to rapidly identify bioactive natural products.